Protocol for SDS-PAGE

1. Prepare the separating and stacking gel solution:

	Stacking gel (4%) for 10 gels	Separating gel (X%) for 10 gels
30% Acr-Bis	3.3ml	1.65*X ml
0.5 M Tris-HCl, pH 6.8	6.3ml	-
1.5 M Tris-HCl, pH 8.8	-	12.5ml
10% SDS	250μL	500μL
ddH2O	15ml	36.75-1.65*X ml
10%APS	12.5μL Each gel	25μL Each gel
TEMED	2.5μL Each gel	2.5μL Each gel

Note: APS and TEMED must be added right before each use.

2. Pipet appropriate amount of separating gel solution (5 ml in our experiment) into the gap between the glass plates set on the casting stands, fill in ethanol (~500 μL) into the gap to make the top of the separating gel be horizontal. Wait for 40 min to gelate.

3. Discard the water and pipet in stacking gel until an overflow. Insert the well-

forming comb without trapping air under the teeth. Wait for 20-30min to let it gelate.

4. Make sure a complete gelation of the stacking gel and take out the comb. Take the glass plates out of the casting frame and set them in the cell buffer dam. Pour 1x running buffer (25 mM Tris-HCl, 200 mM Glycine, 0.1% (w/v) SDS, pH 8.3) into the inner chamber.

5. Prepare the protein sample: Mix the protein with 5x loading buffer (10% (w/v) SDS, 10 mM β-mercaptoethanol, 20 % (v/v) Glycerol, 0.2 M Tris-HCl, pH 6.8, 0.05% (w/v) Bromophenolblue) and hit for 5 min at 95℃.

6. Load prepared samples into wells and set an appropriate volt (140V for stacking gel and 190V for separating gel in our experiment) to run the electrophoresis.

7. Stop running when bromophenolblue reaches (or exceeds) the foot line of the glass plate.